Osteoblast-like cell adhesion on bioactive glasses: surface reactions and resistance to trypsinization

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A preliminary study was initiated to identify the early stages of apatite formation on bioactive glass substrates. Several bioactive glasses are being examined to determine the effects of glass composition on the early processes of apatite nucleation and growth. The objective of this project is to improve osseointegration of implant materials for dental and orthopedic applications. The high spatial resolution of the FTIR system on beam line 1.4.3 has allowed us to examine small isolated features on the glass surfaces. We are continuing this effort during the next experimental period by examining the progression of apatite formation with increasing exposure time to simulated body fluid.

We have developed bioactive glass coatings of Ti alloys that provide good metal adhesion while retaining bioactivity. Two of the glasses (6P1 and 6P8) proved to be suitable substrates for the attachment of osteoblast-like cells. Osteoblast-like cells, when seeded on glass 6P8, showed remarkable resistance to detachment by trypsinization. The purpose of this study was to investigate two possible mechanisms underlying trypsin resistance of MG63 osteoblast-like cells on glass 6P8: 1) trypsin inactivation by solubility products released from glass 6P8 in tissue culture medium; 2) differential protein adsorption on the substrates. Glass discs of the same dimensions ($\varnothing = 12$ mm) were finished through 0.05 μm alumina slurry, cleaned by ultra-sonication in alcohol, sterilized in dry heat at 250° C, and placed in 12-well tissue culture plates (N=5 per material). Human osteosarcoma (osteoblast-like) cells (MG63) were cultured in α–MEM with 10% fetal calf serum and antibiotics. 4×10^5 cells in a 20 μL aliquot were plated on each glass or titanium alloy (Ti6Al4V), as a control. Cells were allowed to settle for 1 hr prior to flooding with medium. After 30 min cells were treated with 1.5 mL of trypsin, either fresh or previously incubated for 1 hr with a disc of glass 6P8. Cells were completely detached from Ti6Al4V at 5 min and glass 6P1 at 10 min. After 15 min cells were still adhering onto glass 6P8, as previously observed. Therefore, cell adherence does not seem to be due to glass 6P8 reactivity products inactivating trypsin. Then specimens of the same two glasses and Ti6Al4V (control) (N=4) were prepared, as described above, and incubated with 2 mL of fetal calf serum for 2 hr at 37 °C. Samples were gently rinsed with PBS to remove weakly adsorbed proteins and desorbed with 10 rinses of 500 μL of 0.1% sodium dodecyl sulphate (SDS). The SDS rinses were collected and analyzed for protein concentration, using a spectrophotometric assay (BioRad Laboratories, and Molecular Devices ν max kinetic microplate reader) with serum albumin as the standard. Data normalized to sample surface area showed significant differences in the amount of protein adsorbed per unit of surface area among substrates (6P8>6P1>Ti6Al4V, one-way ANOVA $p<0.001$). Aliquots of the same samples were fractionated by SDS-polyacrylamide gel electrophoresis, and the proteins visualized by staining with Coomassie blue. The distribution of the bands indicated differential protein adsorption between the bioactive glasses and Ti6Al4V. Glass surfaces were analyzed by Fourier transform infrared spectroscopy (FTIR) before and after the protein adsorption

experiments to identify surface reactions and residual adsorbed proteins. FTIR surface analysis showed that glass 6P8 readily reacted in solution, forming silanols, while glass 6P1 did not. These results indicate that resistance to trypsinization of osteoblast-like cells from glass 6P8 may be due to differential protein adsorption but not to trypsin inactivation. Supported by NIH/NIDCR Grant DE 11289

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